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Customer Number: 000959

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney
Docket No. PKP-006-1

In re the application of Seth Taylor

For: GEL PAD ARRAYS

Assistant Commissioner For Patents
Box Provisional Patent Application
Washington, DC 20231

CERTIFICATION UNDER 37 CFR 1.10

Date of Deposit: January 20, 1998 Mailing Label Number: EM284 255 246US

I hereby certify that this Cover Sheet for Filing Provisional Application (37 C.F.R. §1.51(2)(i)) and the documents referred to as attached therein are being deposited with the United States Postal Service on the date indicated above in an envelope as "Express Mail Post Office to Addressee" service under 37 CFR 1.10 and addressed to the Assistant Commissioner for Patents, Box Provisional Patent Application, Washington, D.C. 20231.

Mark . Russett
Name of Person Mailing Paper

Mark Russett
Signature of Person Mailing Paper

COVER SHEET FOR FILING PROVISIONAL PATENT APPLICATION

Dear Sir:

The accompanying application, entitled GEL PAD ARRAYS, is a provisional patent application under 37 C.F.R. §1.51(a)(2) and §1.53(b)(2).

1. ☒ The name(s) and address(es) of the inventor(s) of this application is/are as follows:

	Last Name	First Name	Middle Initial	Residence
1	Taylor	Seth		Cambridge, MA
2				
3				
4				

2. ☐ This invention was made by an agency of the United States Government or under contract with an agency of the United States Government. The name of the U.S. Government agency and the Government contract number are:

Agency:_____
Contract No.:_____

3. ☒ The following documents are enclosed:

   - ☒ 6 page(s) of specification
   - ☒ 1 sheet(s) of drawings
   - ☒ 1 page(s) of claims
   - ☐ _____ page(s) of power of attorney

4. ☐ A verified statement to establish small entity status under 37 CFR 1.9 and 1.27 is enclosed.

5. ☐ An Assignment of the invention to ______________________ is enclosed. A check in the amount of $40.00 for recording this assignment and a recordation form cover sheet (Form PTO 1595) are also enclosed.

6. ☒ The fee for filing this provisional application, as set forth in 37 CFR 1.16(k), is $150.00 .

   a. ☒ A check for this filing fee is enclosed.

   b. ☐ Charge the filing fee to Deposit Account No. 12-0080. (A duplicate copy of this sheet is enclosed.)

   c. ☐ The filing fee is not being paid at this time.

7. ☒ Please charge any fee deficiencies associated with this filing to Deposit Account No. 12-0080. A duplicate copy of this sheet is enclosed.

8. ☒ Please address all future communications to: **Customer Number: 000959** whose address is:

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Respectfully submitted,

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GEL PAD ARRAYS AND METHODS OF MAKING AND USING THEM

Background of the Invention

Rapid advances in the ability to accurately determine polynucleotide sequences, such as DNAs and RNAs from the genomes of organisms, has made possible the sequencing of huge quantities of polynucleotides. In recent years, the entire genomes of microorganisms, such as *Helicobacter pylori*, have been sequenced.

Traditional sequencing methods have relied on automated sequencing equipment which processes a polynucleotide strand one base at a time. A more recent approach, sequencing by hybridization (SBH), which could potentially increase sequencing throughput, relies on fragmenting a target polynucleotide into short segments; these short segments can be captured, for example on an ordered array of immobilized complementary strands, and the sequences of the individual fragments determined. Alignment of the fragment sequences, typically with the aid of a computer for longer sequences of interest, provides the sequence of the target polynucleotide. See, for example, U.S. Patent No. 5,552,270 to Khrapko *et al.*

Arrays of immobilized polynucleotides have been constructed for use in SBH techniques. However, new types of arrays, and methods for making them, are needed.

Summary of the Invention

This invention features gel pad arrays, e.g., arrays on a support, and methods for making and using them. The arrays can be used for sequencing by hybridization (e.g., where the pads include nucleic acid strands immobilized within the gel matrix), for cell based assays (e.g., where the pads include, or are adjacent to and contacting, living cells), and for other uses which will be apparent to one of ordinary skill in the art.

In one aspect, the invention provides a method for preparing an array of gel pads. The method includes the steps of providing a first gel layer on a substrate; selectively removing portions of the first gel layer to create voids in the first gel layer; providing a second gel in the voids; and removing the first gel layer, such that an array of gel pads is provided.

In another embodiment, the invention provides a gel pad comprising a living cell; or an array of such gel pads. In another aspect, the invention provides a gel pad comprising a first gel layer and a second gel layer adjacent to and in contact with said first gel layer.

Brief Description of the Drawings

Figure 1 depicts gel pads of the invention which include living cells.

Detailed Description of the Invention

This invention provides gel pads and gel pad arrays having a variety of uses, some of ehich are known in the art. The invention also provides methods for making the gel pads and gel pad arrays of the invention.

The term "gel pad" is known in the art and as used herein refers to a discrete portion of a gel disposed on a substarte such as a solid support, e.g., a plastic, glass, or metal substrate. The substrate can be any support suitable for supporting a gel pad, and can be rigid (e.g., a glass or plastic plate or sheet) or flexible (e.g., a tape), transparent (e.g., for performing optical measurements through the pad and substrate) or opaque. The properties of the solid support can be readily selected for use in any particular application. In preferred embodiments, the solid support is substantially non-reactive under conditions used to perform an assay or test procedure with the gel pad or gel pad array.

A gel pad can have any convenient dimension for use in a particular assay. In preferred embodiments, a gel pad is thin enough, and porous enough, to permit rapid diffusion of at least certain reaction components into the gel pad when a solution or suspension is place din contact with the gel pad. For example, in one embodiment, a gel pad array for use in sequencing by hybridization permits polynucleotide fragments from a sample mixture to diffuse (within a conveniently short time period) into the gel pads and hybridize to oligonucleotide capture sequences disposed within the gel pads. In certain preferred embodiments, a gel pad (e.g., in an array of gel pads) has a thickness of at least about 1, 5, 10, 20, 30, 40, 50 or 100 microns. In certain preferred embodiments, a gel pad (e.g., in an array of gel pads) has a thickness of less than about 1 millimeter, 500 microns, 200, 100, 50, 40, 30, 20, 10, 5, or 1 microns.

In one aspect, the invention provides methods for making gel pads and gel pad arrays. In certain preferred embodiments, gel pads and gel pad arrays can be conveniently prepared by use of "intelligent gels." Intelligent gels are gels which have properties which change in response to changes in external conditions (for descriptions of certain intelligent gels, see, e.g., Kajiwara et al., "Synthetic Gels on the Move", *Nature*, vol. 355, pp. 208-209 (1992); Kwon et al., "Electrically Erodible Polymer Gel for Controlled Release of Drugs", Nature, vol. 354, pp. 291-293 (1991); Suzuki et al., "Phase Transition in Polymer Gels Induced by Visible Light", *Nature*, vol. 346, pp. 345-347 (1990); Osada et al., " Intelligent Gels" , *Scientific American*, pp. 82-87 (1993); R. Dagani, "Intelligent Gels," *Chem. Eng. News.*, June 9, 1997). Examples of intelligent gels include gels which become softer or firmer (e.g., solidify or liquefy) in respose to changes in temperature, salt concentration (e.g., ionic strength), pH, exposure to radiation (e.g., ultaviolet (UV) radiation), presence or absence of a selected metal ion, and the like. For example, a copolymer of poly(acrylic acid) and poly(N-isopropylacrylamide) has been reported to be temperature-sensitive, swelling at lower temperatures and collapsing at higher temperatures (Tanaka et al., *Faraday Discuss.* 101:201 (1995)). One of ordinary skill in the art will be able to select an intelligent gel with the desired properties for a selected application using no more than routine experimentation. In certain preferred embodiments, an intelligent gel for use in the present invention is responsive (e.g., liquefies) in response to an increase in temperature or irradiation with ultraviolet light.

In an illustrative embodiment, an intelligent gel can be used to prepare a gel pad array. The gel pads can comprise an intelligent gel, or the intelligent gel can be used as a form or mold to prepare a gel pad array. For example, in one embodiment, an intelligent gel which liquifies in response to UV irradiation is cast is in a thin film on a substrate such as a glass plate. The gel can incorporate reagents, such as polynucleotide probes for capturing fragments of DNA from a solution; alternatively, such reagents can be added after the array has been formed. The gel is allowed to cool and solidify. The gel layer is then masked, e.g., with a mask such as is conventionally used in photolithography; the mask protects gel portions in an array configuration on the substrate (e.g., a 100 x 100 array of gel pads). The masked gel layer is exposed to ultraviolet light. The exposed portions of the gel liquify and are poured off or washed off with a suitable solvent, without disturbing the array. After irradiation and removal of the mask, an array of gel pads is obtained.

Alternatively, an intelligent gel can be used as a mold or form for preparing a gel pad array. An intelligent gel which is temperature-responsive is cast on a substrate. The gel layer is then exposed to a laser, which is rasters over the gel layer and irradiates selected gel portions in the configuration of an array. The portions of the gel pad which are exposed to the laser source are heated and become liquefied; the liquefied portions are removed, e.g., by gentle washing. (The gel layer could be selectively heated by other means, such as an array of heated wires or probes which are brought near to, or into contact with, the surface of the gel layer.) The gel layer now has an array of "holes" formed by removal of the gel portions exposed to the laser source. These "holes" can be filled with a second gel (which can be a different intelligent gel or a conventional gel, such as polyacrylamide); the second gel is permitted to solidify, forming an array of gel pads within the intelligent gel layer. The slide is then heated (e.g., by placing the substrate in a warming bath or a warming oven) to liquefy the intelligent gel layer, which is then removed by washing or pouring off the liquefied material. An array of gel pads remains on the substrate and can be further processed, if desired.

It will be appreciated that the methods of using intelligent gels to prepare gel pad arrays will have many applications. The mild conditions employed can be tailored to the preparation of a wide variety of intelligent and conventional gel pad arrays, preferably without degradation of sensitive reagents, such as polynucleotide probes, which may be present in the gel layer.

Furthermore, the use of intelligent gels in gel pad arrays provides additional advantages. For example, an intelligent gel pad can be provided which swells in reposne to a change, such as the presence of an analyte of interest. For example, an intelligent gel which swells in response to pH changes in provide in a gel pad on a support. The gel pad includes glucose oxidase. The reaction of glucose oxidase with glucose produces gluconic acid, lowering the pH of the gel. Thus, in the presence of glucose in a sample solution which is brought into contact with the gel pad, the gel pad will shrink. A gel pad can be provided

adjacent to a piezocrystal, such that changes in gel pad swelling produce a piezoelectric signal, which can be detected and correlated with the glucose concentration.

Gel pad arrays can also be prepared by treating the surface of the substrate to create a pattern of alternating hydrophobic and hydrophilic sites on the surface. For example, a glass surface can be silated with a conventional silating reagent to prepare a patterned surface having hydrophobic and hydrophilic portions. A gel, such as an intelligent gel, is then poured onto the surface. A hydrophobic gel will be repelled by a hydrophilic surface, while a hydrophilic gel will be repelled by a hydrophobic surface. A patterned surface can be used to urge the liquified gel into a pre-selected pattern on the substrate, thereby forming a gel pad array.

In another embodiment, a gel pad (e.g., in an array) can be prepared through the use of a derivatized monomer unit, followed by formation of the gel pad by polymerization of the monomer. For example, acrylic acid can be readily derivatized with a polynucleotide (e.g., a probe for use in SBH); for example, a polynucleotide can be coupled to acrylic acid through the use of a conventional coupling reagent such as dicyclohexylcarbodiimide (DCC) (or a water-soluble derivative thereof such as 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, EDC). A spacer or linker moiety can be used to increase the distance between the acrylate monomer and the polynucleotide, if desired, e.g., to increase mobility of the polynucleotide in the polymer). The resulting acrylic ester of the polynucleotide can then be disposed in an array format on a substrate, e.g., by dispensing a solution of the acrylic ester through a nozzle or array of nozzles (such as conventional piezoelectric ink-jet printing nozzles). Alternatively, an array format can be provides by using a cast or mold. The array of droplets is then polymerized *in situ* to provide an array of gel pads which incorporate a polynucleotide covalently bound to the gel polymer.

The invention also provides multi-layered gel pad constructs. For example, in one aspect, the invention provides a gel pad which comprises at least two gel layers in contact with each other, e.g., a first gel layer on which is disposed a second gel layer, or first gel layer adjacent to and in contact with a second gel layer. A multi-layer gel pad of the invention can have two, three, four, or more layers, although greater numbers of layers will generally require more effort to prepare. The multi-layer gel pads of the invention can be configured to provide a variety of functions. For example, a first gel pad layer can include a polynucleotide (e.g., a probe for performing SBH) within the first gel matrix. A second gel layer can be disposed over and covering the first gel layer; the second gel layer can be a gel having an effective pore size small enough to prevent the diffusion of high-molecular-weight substances, such as proteins. The second layer thus serves as an effective barrier to prevent diffusion of substances, e.g., proteins, from a sample solution into the first gel layer, or from the first gel layer into solution. The multi-layer gel pad can prevent interference from sample constituents, or can prevent the loss of valuable components from the first gel layer.

In another embodiment, a first gel layer can be formed with low ionic strength, e.g., an ionic strnegth lower than the ionic strength of a sample solution to be applied to the gel pad array. A second, protective or filtering gel layer covers and encapsulates the first gel pad layer. The low ionic strength of the first gel layer promotes osmotic movement of sample components into the first gel layer, thereby increasing the sensitivity of the first gel layer for a sample component of interest.

A multi-layer gel pad can be constructed by methods known in the art for the preparation of single-layer gel pads, or by the methods described herein. It will be appreciated that in certain embodiments, it is preferred to maintain registration between the layers of the multi-layer gel pad, e.g., in certain embodiments, it is preferred to place a second gel layer directly atop a first gel alyer. The use of a mold or form can be useful in this embodiment, because molds can provide good registration between layers.. A particularly useful method for preparing a multi-layer gel pad array is the intelligent gel "molding" or "forming" layer methodology described above.

In still another embodiment, the invention provides gel pads which include living cells (referred to herein as "cell pads"). In one embodiment, the gel pad of the invention is a multi-layered gel pad, having a first layer without cells, and second layer which includes cells (e.g., bacterial or eukaryotic cells). (Alternatively, cells can be grown on top of a gel layer, without being immobilized within a second gel layer). Exemplary embdiments are shown in Figure 1. Figure 1A depicts a first gel layer disposed adjacent a second, cell-containing gel layer. Figure 1B depicts cells immobilized in a second gel layer which encapsulates a first gel layer. Figure 1C shows cells maintained on the surface of a gel layer. The cells can be maintained in culture. This embodiment, provides a useful assay format for performing cell based assays in an array format. For example, the first gel layer could include detection means for detecting the presence (or absence) of a cell constituent (such as DNA) or a product of cellular metabolism (such as proteins, or products of transcription). For example, the cells in one layer can secrete molecules, such as growth factors, which can be monitored by the use of capture molecules in another layer of the multi-layer gel pad. The cells can also be lysed and cellular components measured. Thus, the response of the cells to a stimulus, such as addition of a growth factor, a toxin, a drug, or the like, can be monitored in a convenient and easily handled format.

Cell pads can also be configured to permit cells in one pad to secrete molecules which influence the growth of other cells in adjacent pads, e.g., an autocrine system. Thus, complex cell-beased assays can be reduces to microscale format.

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, numerous equivalents to the specific procedures described herein.

Such equivalents are considered to be within the scope of this invention and are covered by the following claims.

The contents of all publications and patent applications described herein are hereby incorporated by reference.

Other embodiments are within the following claims.

What is claimed is:

1. A method for preparing an array of gel pads, the method comprising:
providing a first gel layer on a substrate;
selectively removing portions of the first gel layer to create voids in the first gel layer;
providing a second gel in the voids; and
removing the first gel layer, such that an array of gel pads is provided.

2. A gel pad comprising a living cell.

3. An array of the gel pads of claim 2.

4. A gel pad comprising a first gel layer and a second gel layer adjacent to and in contact with said first gel layer.

Abstract

Gel pads and gel pad arrays, and methods for making and using them, are disclosed. The gel pads preferably comprise an intelligent gel.

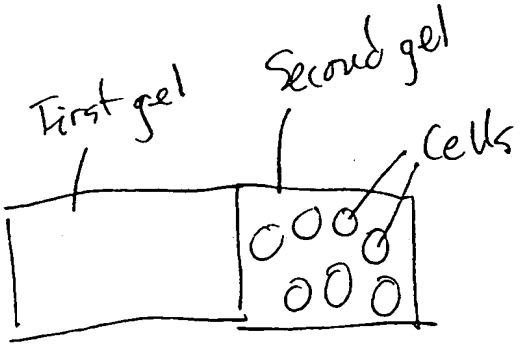

Fig. 1A

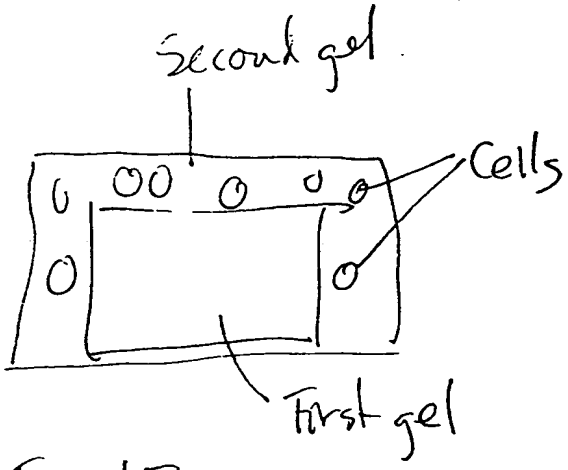

Fig. 1B

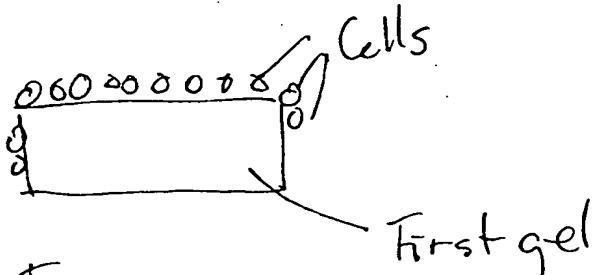

Fig. 1C